APPLIED MICROBIOLOGY, Nov., 1965 Vol. 13, No. 6
 Printed in U.S.A.

Geosmin, an Earthy-Smelling Substance Isolated from Actinomycetes

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Received for publication 17 June 1965

ABSTRACT

GERBER, N. N. (Rutgers, The State University, New Brunswick, N.J.), AND H. A. LECHEVALIER. Geosmin, an earthy-smelling substance isolated from actinomycetes. Appl. Microbiol. **13**:935-938. 1965.—Geosmin, an earthy-smelling substance, has been isolated from several actinomycetes. Production of 1 mg per liter of whole broth was obtained from *Streptomyces griseus* LP-16. After preliminary separations, pure geosmin was isolated in milligram amounts by gas chromatography. Geosmin is a neutral oil, with an approximate boiling point of 270 C, which contains carbon and hydrogen, but no nitrogen. It undergoes a reaction with acid to give odorless argosmin, a neutral oil, with an approximate boiling point of 230 C, which contains only carbon and hydrogen. Specific rotation and ultraviolet- and infrared-absorbtion spectra were determined for both.

Freshly plowed soil has a typical odor which was undoubtedly detected even by primeval men and extolled in all tongues by bucolic poets. In the 19th century, the odor of the soil was subjected, for the first time, to scientific scrutiny. Berthelot and André (1891) noted that the substance responsible for the typical earthy odor of the soil could be extracted from soil by steam and was probably neutral. As microorganisms began to be grown in pure culture, it became obvious that some of them, mainly actinomycetes, produced an earthy odor. In addition, these organisms were suspected of imparting taints to bodies of water and their inhabitants. Thaysen (1936) and Thaysen and Pentelow (1936) obtained ether-soluble extracts of actinomycetes which had a manurial odor at high concentrations, but which, upon dilution, became earthy-smelling. A live fish placed in water containing minute amounts of the odoriferous substance picked up the taint and stored it in its flesh. This absorption was rapid and took place either through the gills or the mouth, not through the skin. The elimination of the taint was slow and required keeping the fish alive for a few days in pure running water. Numerous investigations have shown the role played by actinomycetes in giving water undesirable odors and tastes, and methods of control have been studied (Silvey and Roach, 1956; Hoak, 1957; Bartholomew, 1958).

Romano and Safferman (1963) grew an odoriferous strain of *Streptomyces griseoluteus* in a glucose-glutamate-yeast extract-salt medium. After proper incubation, about 90% of the odoriferous material could be recovered in the first 10% of the distillate from the culture medium. Further purification could be achieved by ether extraction of the distillate, or by adsorption of the distillate on activated carbon followed by chloroform extraction. The pure odoriferous material was not obtained by these authors, but their concentrates could be diluted 1 billion-fold and still retain the characteristic odor.

Gaines and Collins (1963) studied the metabolites of *S. odorifer* and concluded that the earthy odor might be due to the production of a combination of trivial compounds such as acetic acid, acetaldehyde, ethyl alcohol, isobutyl alcohol, isobutyl acetate, and ammonia. They emphasized that other constituents contributing to the odor might also be produced.

Our study shows that one specific compound having an earthy odor can be found among the metabolites of numerous actinomycetes. The physical properties of this compound, geosmin (from the Greek "ge" = earth and "osme" = odor) have been determined. With aqueous acid, geosmin is transformed to "argosmin" (from the Greek "argos" = inactive and "osme" = odor) which has similar, although not identical, properties, but no odor.

MATERIALS AND METHODS

Organisms. The organisms with IMRU numbers are from the collection of the Institute of Micro-

biology, Rutgers University. Other numbers refer to the collection of H. A. Lechevalier.

Media and culture conditions. The yeast extract-glucose (YD) medium used consisted of 10 g of yeast extract (Difco) and 10 g of cerelose per liter of tap water. The *p*H was adjusted to 7.0 to 7.2. When calcium carbonate was added, it was at the level of 100 mg per flask.

Soy bean meal (SBM) was prepared by boiling 20 g of soy bean meal and 5 g of BYF no. 50X (a fraction of autolyzed brewers yeast sold by Amber Laboratories, Inc., Milwaukee, Wis.) for 10 min with water and then filtering it through cheesecloth. To this filtrate were added 5 g of sodium chloride and 10 g of cerelose, and the mixture was diluted to 1 liter with tap water; the *p*H was adjusted to 7.5, and the medium was dispensed into flasks which already contained 100 mg of calcium carbonate per 100 ml of medium.

Pablum medium consisted of 60 g of Pablum mixed cereal (Pablum Products, Evansville, Ind.) in 1 liter of tap water, which had been boiled for 10 min and then dispensed.

All media were autoclaved for 15 to 20 min at 115 psi and 121 C.

The organisms were maintained on YD slants except for the *S. fradiae* strains, which were maintained on potato-carrot-agar (Segretain, Drouhet, and Mariat, 1958). All were incubated at 28 C until well grown and were then stored at 5 C for 2 to 3 months. When testing the organisms for geosmin production, a 48-hr shake flask inoculum grown on YD (75 ml per 250-ml flask) was subcultured at 5% into various media (75 ml per 250-ml flask) and incubated at 215 rev/min at 28 C on a rotary action shaker (model V, New Brunswick Scientific Co., New Brunswick, N.J.). For large-scale production of geosmin from *S. griseus* LP-16, an identical inoculum was subcultured into SBM medium (300 ml per 2-liter flask) and incubated at 28 C on a reciprocal shaker (model 5713, New Brunswick Scientific Co., New Brunswick, N.J.) at 80 strokes per min for 3 days. With *S. antibioticus* IMRU 3720, large-scale production was carried out similarly with Pablum medium and incubation for 7 days.

Preliminary separation of geosmin. All whole broths were distilled at atmospheric pressure until 20% of their volume had been collected as distillate. The distillates were about half-saturated with sodium chloride (20 g per 100 ml) and extracted twice with 10 to 20% (v/v) of methylene chloride. After drying, the methylene chloride solutions were concentrated by distillation or by a stream of warm air.

Preparation of argosmin. Argosmin was prepared by adding 0.5 ml of concentrated hydrochloric acid to one batch of crude geosmin (from 9 liters of whole broth) in 10 ml of methylene chloride. After standing for 3 to 5 days at room temperature, tightly stoppered, the solution was reduced to 5 ml in a stream of warm air and applied to a 10-g column of Mallinkrodt silicic acid powder, 100 mesh. With methylene chloride, argosmin was eluted rapidly, usually in the second 10-ml fraction; most impurities were retained on the column.

Gas chromatography. Gas chromatography was with an F & M (Avondale, Pa.) model 700 dual-column instrument with a thermal conductivity detector. The concentrated methylene chloride solutions were analyzed by gas chromatography with a 4 ft by ⅛ inch (122 by 0.3 cm) column of 10% SE-30 on Diatoport W 60–80 mesh. This is a nonpolar column which separates compounds roughly in order of their volatility. The conditions for satisfactory separation (detector, 260 C; injection port, 300 C; column, 155 C programmed at 4 C per min; bridge current, 150 mv; carrier gas helium at 50 ml/min) were such that naphthalene and anthracene had retention times of 1.7 and 10.6 min, respectively. This column had about 900 theoretical plates. A second column 6 ft (183 cm) long but otherwise identical had about 1,250 theoretical plates under the same conditions. The efficiencies of both columns declined as they were used for preparative separations of geosmin.

Pure samples of the osmins were collected from the exit port of the gas chromatograph in specially cleaned Pyrex tubing (8 cm by 2 mm internal diameter) which had a slight constriction in the middle. The glass tube was attached with a 1-cm piece of new latex tubing only during the time that the desired peak appeared on the recorder. Chromatography conditions were the same as above except that, to insure complete separation of peaks at the heavier loading, a column temperature of 100 C programmed at 4 C per min was used. When enough had been collected, as judged by weighing the tube, one end was sealed. Then the liquid was centrifuged to the closed end and the tube was sealed at the constriction.

Properties of the osmins. To determine the approximate boiling points of the osmins, they were compared on the gas chromatograph under constant, isothermal conditions with a series of nonpolar liquids of known boiling points. Ultraviolet absorbtion of 0.1% solutions in chloroform were measured on a Cary model 14 recording spectrophotometer; infrared spectra of 3 to 10% solutions in chloroform were determined on a Perkin-Elmer Infracord. Rotations were taken on 0.5% solutions in chloroform in a 1-decimeter tube. Elemental analyses were made by George Robertson, Florham Park, N.J.

RESULTS AND DISCUSSION

S. griseus LP-16 was selected at the beginning of this investigation because of the strong earthy odor of its slant cultures. Methylene chloride extracts of whole broths or distillates of these broths were analyzed by gas chromatography as explained above, and the substance responsible for the characteristic earthy odor came out of the exit port at the same time that a sharp symmetrical peak with a retention time of 4.0 min appeared on the recorder. It soon became

TABLE 1. *Geosmin production by various actinomycetes as detected by gas chromatography*

Organism	Production medium	Day of maximal geosmin production	Yield of geosmin*
			μg/ml
Streptomyces griseus LP-16	YD + $CaCO_3$	2	0.94
	SBM	2	1.25
	Pablum	4	0.03
S. antibioticus IMRU 3720	SBM	4	0.28
	Pablum	6, 7	0.78, 1.75
S. antibioticus IMRU 3491	Pablum	4	0.46
S. fradiae IMRU 3535	Pablum	6	0.34
S. fradiae IMRU 3535-R7	SBM	4	0.03
	Pablum	4-6	0.1
S. odorifer IMRU 3334	YD + $CaCO_3$	4	0.25
	SBM	2-4	0.25
	Pablum	2-6	0.37

* Quantitative gas chromatographic determinations: 1 μg of pure geosmin = a peak with an area of 8 mm^2 under the conditions used.

evident that geosmin was present in broths which did not have an earthy odor, its characteristic smell being covered by other compounds. Gas chromatography, however, permitted the resolution of the mixtures, and the geosmin odor was detected at the exit port after 4.0-min retention time.

A small survey of various actinomycetes for geosmin production gave the results shown in Table 1. *S. griseoluteus* IMRU 3718 grown on the three production media indicated gave no geosmin. This may be due to differences in the strains or media employed by Romano and Safferman (1963) and by us, or to a difference of opinion as to what constitutes a typical "earthy" odor.

Yields from the large-scale production of geosmin by *S. griseus* LP-16 (usually 9 liters of whole broth) varied from 0.9 to 1.6 μg/ml of whole broth. These figures were based on the fact that peaks of about 8,000 mm^2 were recorded when 1 mg of geosmin was collected from the gas chromatograph. The retention time of geosmin under the conditions of preparative separation explained above was 13 min. The approximate boiling point based on a comparison of retention times with those of known materials was 270 C.

Pure geosmin in 10% hydrochloric acid stood 4 days at room temperature. Gas chromatography of the methylene chloride extract of the acid solution showed only pure argosmin. Its retention time under conditions of preparative separation was 9.0 min and its approximate boiling point was 230 C.

Geosmin was found to have only end absorption in ultraviolet light; at 300, 260, and 240 mμ, the $E^{1\%}_{1cm}$ values were 3.3, 8.0, and 10.7, respectively. The $[\alpha]^{25}_D$ was −16.5°. The infrared spectrum showed bands at 3.55, 6.00, 6.35 (last two very weak), 6.95, 7.37, 7.87 (broad), 8.6, 8.8 (shoulder), 9.15 (broad), 10.0, 10.3, 10.6, 10.95, 11.35, and 11.8 μ. Also, argosmin had only end absorption in ultraviolet light; at 300, 260, and 240 mμ, the $E^{1\%}_{1cm}$ values were 0.7, 3.4, and 18.5, respectively. The $[\alpha]^{25}_D$ was +29°. The infrared spectrum showed bands at 3.55, 6.35 (weak), 6.95, 7.39, 8.45 (broad), 9.55, 10.15 (broad), 10.65, 11.5, and 12.4 μ. For both osmins, the infrared spectrum indicates the absence of O-H, N-H, or carbonyl groups, and the possible presence of an ethylenic linkage. The ultraviolet spectrum shows that no aromatic rings are present.

Neither geosmin nor argosmin contained nitrogen. Geosmin contained about 79% carbon and 12% hydrogen; argosmin, about 86% carbon and 12% hydrogen. The usual difficulties in analyzing small samples of moderately volatile liquids prevented precise determinations. Argosmin thus appears to be a hydrocarbon. The acid-catalyzed transformation of geosmin to argosmin includes the loss of some element other than carbon, hydrogen, or nitrogen (probably oxygen).

ACKNOWLEDGMENTS

We wish to acknowledge the valuable technical assistance of Eva M. Fekete and Jane Reece. We also wish to thank Mrs. M. P. Lechevalier for valuable advice.

This investigation was supported by Public Health Service grant WP 00515 from the Division of Water Supply and Pollution Control.

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